PATENT SPECIFICATION

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COMPLETE SPECIFICATION

NO DRAWINGS

Multivitamin Compositions

We, MERCK & CO., INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to stable vitamin compositions and methods for preparing such compositions. More particularly, it is concerned with the preparation of vitamin compositions containing a stabilized form of pantothenic acid.

Pantothenic acid, one of the important vitamins of the vitamin B group, is a hygroscopic viscous liquid which is relatively unstable and hence not useful as a source of pantothenic acid activity in pharmaceutical preparations. Generally, the calcium salt of pantothenic acid, which is a relatively high melting solid, is used in pharmaceutical preparations since this salt is more stable than pantothenic acid itself. However, even calcium pantothenate is not especially suitable for use in multivitamin compositions since it is not stable for extended periods in such formulations. It is therefore necessary, for example in the preparation of multivitamin vitamin formulations, to include an excess of calcium pantothenate in order to ensure that the product will have described pantothenic acid activity even after storage for some months.

In accordance with the present invention, it has now been found that pharmaceutical preparations, particularly multivitamin compositions, in which the source of pantothenic activity is pantothenamide are unusually stable and lose little of their pantothenic acid activity even upon extended storage under adverse conditions. Thus, the use of pantothenamide in such preparations avoids the need of including a large excess of pantothenic acid and in addition provides a product having enhanced stability.

In accordance with the invention, it has also been found that when pantothenamide is incorporated in multivitamin preparations such as syrup, capsules, drops and tablets, it is much more stable than salts of pantothenic acid such as calcium pantothenate. This invention therefore provides a convenient method of preparing multivitamin compositions having a stable pantothenic acid component which can be stored for extended periods of time without decomposition.

Pantothenamide is particularly useful as a source of pantothenic acid activity in various multivitamin compositions since such compositions usually have a pH of about 4-5 and the amide is very stable in this pH range. Calcium pantothenate on the other hand, is more unstable at these acidic pH's and is therefore not suitable for use under these conditions.

The following examples illustrate the new compositions and the method of preparing such compositions.

EXAMPLE 1

A liquid multivitamin formulation suitable for dispensing as drops was prepared by procedures known in the art and containing the following ingredients:

	Amount per 0.6 ml.
Vitamin A Palmitate	5000 units
Vitamin D_2	1000 units
Thiamine HCl	1 mg.
Riboflavin	0.8 mg.
Niacinamide	10 mg.
Pyridoxine HCl	1 mg.
Ascorbic Acid	50 mg.

	Amount per 0.6 ml.
Cyanocobalamin (crystalline)	5 meg.
Sorbitol solution 70%	.24 g.
Deionized water	0.06 g.
Polyethylene oxide sorbitan mono-oleate (Trade Mark Tween 80)	0.06 g.
Ethylenediaminotetraacetic acid disodium calcium salt (Trade Mark Sequestrene Na_2Ca)	0.00018 g.
Iron peptonate	0.00071 g.
An antioxidant combination of butylated hydroxy ainisole, propyl gallate and citric acid dissolved in propylene glycol (Trade Mark Tenox II)	0.000018 cc.
Sodium sucaryl	0.0022 g.
Sodium saccharin	0.00024 g.
Methyl paraben (methyl *p*-hydroxybenzoate)	0.00048 g.
Propyl paraben (propyl *p*-hydroxybenzoate)	0.00012 g.
Propylene glycol sufficient to bring solution to 0.6 ml.	

The final pH of the formulation of the pH was about 4. For actual use flavouring agents may also be included.

Two batches of the above-described multivitamin preparation were prepared; one containing d-calcium pantothenate in an amount equivalent to 5 mg. per 0.6 ml. drop and the second d-pantothenamide in an amount equivalent to 5 mg. per 0.6 ml. drop. Samples of the two batches so prepared were subdivided into 10 ml. amber screw cap bottles, flushed with nitrogen, sealed with natural polyethylene polyseal caps and stored at room temperature, 40°C and 45°C. The stored preparations were assayed at intervals for pantothenic acid content and the results recorded in the following table:

Preparation	d-Calcium Pantothenate						d-Pantothenamide					
Storage Temperature	R.T.*		40°C.		45°C.		R.T.*		40°C.		45°C.	
Assay	mg/ml	% Loss	mg/ml	% Loss	mg/ml	% Loss	mg/ml	% Loss	mg/ml	% Loss	mg/ml	% Loss
Initial	4.93		4.93		4.93		5.03		5.03		5.03	
2 Months	—	—	2.80	43	2.13	57	—	—	5.08	0	5.07	0
3 Months	4.03	18	1.73	65	1.09	78	4.96	1.4	3.6	28	3.12	38

* Room Temperature

Thus, in this multivitamin preparation the d-pantothenamide is much more stable upon storage than d-calcium pantothenate.

EXAMPLE 2

Uncoated multivitamin tablets were prepared in accordance with methods known in this art. The composition of the tablets was as follows:

Ingredient	*Amount per Tablet*
Cyanocobalamin in Mannitol	5.0 mg.
Vitamin A and D Crystalets	44.0 „
Thiamine Mononitrate	6.25 „
Riboflavin	10.8 „
Niacinamide	52.5 „
Pyridoxine HCl	1.1 „
Ascorbic Acid	70.8 „
Sodium Ascorbic Acid	106.1 „
Folic Acid	5.3 „
Lactose	50.0 „
Magnesium Stearate	4.5 „
d-Calcium Pantothenate	5.1 „

A second batch of uncoated multivitamin tablets was similarly prepared containing 5.0 mg. of pantothenamide per tablet in place of the d-calcium pantothenate.

The stability of the pantothenic acid components in the two batches of tablets was then compared by assaying the tablets after storage (1) at 50°C. at 50% relative humidity for three weeks, (2) at room temperature for two months, (3) at 45°C. for two months, (4) at 45°C. for three months, and (5) at room temperature for 29 months. The results are shown in the following table:

Storage Data	d-Calcium Pantothenate		d-Pantothenamide	
Temp./Time	mg/cc	% Loss*	mg/cc	% Loss*
Initial	4.2		4.95	
50°C/50% RH 3 wks.	1.95	-54	3.61	-28
45°C/2 months	3.45	-18	4.52	-9
RT/3 months	4.35	0	4.88	-1
45°C/3 months	3.29	-22	4.43	-10
RT/29 months	2.98	-30	4.69	-5

RT is Room Temperature
RH is Relative Humidity
* % Loss computed from Initial Assay

The stability of the pantothenamide component in the multivitamin tablets is strikingly illustrated in the results of the storage for 29 months at room temperature, the pantothenamide preparation losing only 5% of its original activity whereas the d-calcium preparation lost 30% of its original activity.

EXAMPLE 3

A typical multivitamin syrup was prepared in accordance with procedures known in this art. This composition of the vitamins in this syrup was as follows:

Ingredient	*Amount per 5 ml.*
Vitamin A Palmitate	5000 units
Vitamin D_2	1000 units
Riboflavin	1.2 mg
Niacinamide	20.0 mg.
Thiamine HCl	3.0 mg.
Pyridoxine HCl	1.0 mg.
Vitamin B_{12} in Mannitol	5.0 mcg.
Ascorbic Acid	50 mg.
Sodium Ascorbate	50 mg.
d-Calcium Pantothenate	25 mg.
Methyl paraben	0.004 g.
Propyl paraben	0.001 g.
Iron peptonate	0.0013 g.
"Sequestrene Na_2Ca"	0.004 g.
"Tenox II"	0.00016 g.
Enzyme converted corn syrup (Trade Mark Veltose 165)	4.1 g.
Sucrose	.75 g.
Sorbitol solution (70%)	0.2 g.
Acacia	0.01 g.
"Tween 80"	0.01 g.

Deionized water sufficient to make 5 ml.

A second syrup was prepared in the same way containing 25 mg. per 5 ml. of pantothenamide in place of the d-calcium pantothenate.

The two syrups were then subdivided into

10 ml. amber screw cap bottles, flushed with nitrogen, sealed with natural polyethylene polyseal caps, stored at 40 and 45°C, and assayed for pantothenic acid activity periodically.

After four months at 40°C the syrup containing d-calcium pantothenate showed 85% loss of pantothenic acid activity, whereas the pantothenamide containing syrup showed only 6% loss of activity. After four months at 45°C., the d-calcium pantothenate lost all its pantothenic acid activity whereas the d-pantothenamide formulation lost only 10% of its original pantothenic acid activity.

STABILITY TEST AND ASSAY

The stability of pantothenamide and d-calcium pantothenate were compared at concentrations of 3.0 mg/ml in phosphate-citrate buffers at about pH 4 and 5 preserved with methyl p-hydroxybenzoate and propyl p-hydroxybenzoate. The buffered solutions were assayed periodically for pantothenic acid content and the results tabulated in the following table.

pH	Storage Time	d-Calcium Pantothenate				d-Pantothenamide			
		RT		45°C.		RT		45°C.	
		mg/cc	% Loss*	mg/cc	% Loss*	mg/cc	% Loss*	mg/cc	% Loss*
4	Initial	2.79	—	2.79	—	3.02	—	3.02	—
	1 month	—		1.59	-43	—		3.09	0
	1.5 month	—		1.42	-49	—		3.04	0
	2 months	2.70	-3	1.13	-59	2.99	0	3.03	0
	6 months	2.77	0			3.12	0		
	12 months	2.23	-20			3.11	0		
5	Initial	3.27	—	3.27	—	3.11	—	3.11	—
	1 month	—		2.78	-15	—		2.94	-5
	1.5 month	—		2.00	-39	—		2.97	-4
	2 months	3.21	-2	1.98	-39	3.05	-2	2.92	-6
	6 months	2.96	-10			3.01	-3		
	12 months	2.27	-31			3.07	-1		

* % Loss computed from Initial Assay

The assay method used for the determination of calcium pantothenate and pantothenamide involved the hydrolysis of these substances to β-alanine and alanine amide, and the colorimetric determination of these latter products via the ninhydrin reaction for amino acids. This assay was carried out as follows:

Reagents:

1. Dowex 50-X4 (H-type) 100-200 mesh
2. Florisil (60-100 mesh)
3. Phenol solution—dissolve 80 g. of reagent grade phenol in 20 cc. absolute ethanol with gentle heating. Shake after cooling, with 1 g. Dowex 50-X4, for 20 minutes, allow to settle and decant solution.
4. Cyanide-pyridine reagent—2 cc. of a 0.01M solution KCN (freshly prepared) are diluted to 100 cc. with ammonia-free pyridine (prepared by shaking 100 cc. pyridine with 1 g. Dowex 50-X4 [H type] 20 minutes).
5. Ninhydrin solution—dissolve 0.5 g. 1, 2, 3-triketohydrindene in 10 cc. absolute ethanol.

("Dowex" and "Florisil" are Trade Marks)

Procedure: The sample is prepared by dissolving a weighed quantity of a finely powdered sample or by diluting a measured volume of liquid to an appropriate volume. An aliquot of this solution, containing between 2-3 mg. of pantothenates or pantothenamide in not more than 25 cc. H_2O is placed on a column of about 12 mm. diameter and 30 cm. long fitted with a stopcock. The column is prepared by placing a small pledget of glass wool in the bottom, adding about 5 cm. Florisil, pledget of glass wool, 3 cm. Dowex 50-X4, glass wool. Thoroughly wash the column with H_2O before adding sample.

The solution is regulated to a flow at a rate of about 0.5 cc./minute until no liquid remains on top, into a 100 cc. volumetric flask. The column is then rapidly eluted with enough water to make a volume of about 40 cc. in the flask.

Add 2.5 cc. of 0.5N NaOH and heat in boiling water bath for 1 hour to ensure complete hydrolysis.

Cool, add one drop of phenolphthalein TS, and titrate with 1N H_2SO_4 to a colorless end point. Add 2 drops in excess.

Pipette 50 cc. of absolute ethanol into the flask, mix and bring to volume with H_2O.

Place 2 cc. of this solution into a 10 cc. volumetric flask, add 1 cc. of phenol reagent, swirl, add 1 cc. of KCN-pyridine reagent, swirl, and heat 1 minute (timed) in a boiling water bath. CAUTION—temperature is very critical and must be adhered to. Swirl during heating.

Add 0.2 cc. ninhydrin reagent, stopper the flask securely by twisting, and heat 5 minutes in the water bath.

Cool to room temperature and dilute to volume with 60% V/v ethanol.

A standard containing a known quantity (2-3 mg.) of pantothenate or pantothenamide is placed on the same type column as used for the sample and subjected to the same procedure.

A reagent blank (2 cc. of 60% V/v ethanol) is color developed in the same manner as sample and standard.

The photometer is set at 0 absorbance at 570 mu. with the reagent blank and readings of sample and standard are taken.

Calculation:

$$\frac{OD_?}{OD_{std}} \times \text{Conc. STD in mg.} \times \text{dilution factor} = \text{mg. in sample}$$

In assaying of syrups, a portion of syrup equivalent to 15 mg. of calcium pantothenate or 30 mg. of pantothenamide is pipetted into a 25 ml. volumetric flask and made to volume by rinsing the pipette with water. A 10 ml. portion of the solution is added to a 50 ml. centrifuge tube containing 8 g. of ammonium sulfate. The tube is capped shaken for five minutes. 20 ml. of benzyl alcohol is then added and the tube is shaken again for 15 minutes. The tube is centrifuged and 10 ml. of the benzyl alcohol extract removed and added to second 50 ml. centrifuge tube containing 10 ml. of toluene. 15 ml. of water is pipetted into the tube and the mixture shaken vigorously for 15 minutes. The tube is centrifuged and a 10 ml. portion of the water layer is removed and placed on the resin column described above. A standard is treated simultaneously exactly as described above.

WHAT WE CLAIM IS:—

1. A multivitamin composition comprising essential vitamins and pantothenamide.
2. The composition of Claim 1 in the form of a syrup.
3. The composition of Claim 1 in the form of a tablet.
4. The composition of Claim 1 in an encapsulated form.
5. The method of preparing multivitamin compositions which comprises incorporating pantothenamide in such compositions.
6. A composition according to any one of Claims 1-4, containing as the essential vitamins, a plurality of the essential vitamins mentioned hereinbefore in the Examples.
7. A composition according to Claim 1, substantially as hereinbefore described in any one of the Examples.

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